SOME EFFECTS OF STIMULATING GANGLION CELLS.

Preliminary Communication.

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The aim of this series of experiments has been to ascertain to what extent changes due to the functional activity of the nerve cell can be seen by aid of the microscope. The work is based on the fundamental idea in all cell activity, viz. that in the resting state the cell elaborates highly complex compounds, and that these break down to yield the energy by which the cell does its work. These processes have been studied and successfully demonstrated by others in certain gland cells. We applied similar methods to their study in nerve cells; thinking it would be strange if they should prove undemonstrable in cells so large, so definitely characterized, and which stand in so vital a relation to the energy of the animal body.

For this purpose we have used the posterior root ganglia. Fifteen experiments were made on frogs. One, the last experiment, was made on a cat.

The preliminary question as to the minute structure of the ganglion demanded attention at the outset. Mr. Nelson, working under the direction of Dr. Birge at the University of Wisconsin, counted the fibres in the

posterior root and the cells of the corresponding ganglion of the frog, expecting to find, as Birge had found for the anterior root and motor cells, one cell for each fibre. He counted about ten ganglia and, allowing 2 to 4 per cent for error in counting, found *two* nerve cells to the fibre. This would indicate a more complex structure of the ganglion than Ranvier supposed. To study this point we teased the ganglia, using a fine jet of water instead of needles, and obtained preparations which we think demonstrate the following points:

1. Typical bipolar cells do occur, two having been found.

2. The axis cylinder of the process is often seen to divide and enter the cell as a spiral and a straight fibre.

3. At the angles of the "T" the axis cylinder of the cell process may be seen to divide and pass both ways in the nerve fibre, of which it *seldom* forms the *whole* of the axis cylinder.

4. Two cells, in a number of cases, have been found to unite their processes, not necessarily as a cell junction, but to aid in making up the axis cylinder of the same nerve fibre.

If two cells are connected with a fibre in this way, we see no reason why more may not be. So that we may conclude, whatever the number of cells in the ganglion, that our stimulus applied to the nerve trunk will reach them all. They probably function as bipolar cells.

Stimulation Experiments.

Results of these may be briefly summarized.

Four experiments, on frogs, in which curare was used, gave no decisive results.

Five experiments, in which the circulation was disturbed, *i. e.* where the frogs were bled or the capsules

of the ganglia torn off with a view to prevent rejuvenation of the cells, gave unsatisfactory results. See Table I.

Before referring to the tables it should be stated that several observers arrived independently at the conclusion that the nuclei of the stimulated cells looked smaller than those of the unstimulated. This led to the series of measurements given in the subjoined tables. The nuclei were measured, long and short diameters, in sets of one hundred each; fifty stimulated and fifty unstimulated being taken from as nearly corresponding sections of the two ganglia as possible. The measurements were made to the nearest μ under a magnifying power of Leitz Oc. 3, Obj. 7. (= 600 diameters). The diameters were put down in series, then averaged, and this average is given in the tables.

TABLE I.

Frog No. 8. Bled. Stimulated 7 hours; five minutes of stimulation alternating with five minutes of rest.

One set of 100 nuclei. Ganglia hardened in corrosive sublimate.

AVERAGE DIAMETERS IN μ.

	Long.	Short.	Mean.
Resting.....	14.41	10.09	12.25
Stimulated	13.80	10.21	12.00

Staining, structure of protoplasm, etc., not distinguishable.

One experiment, in which the ganglia were suspended in normal salt solution while being stimulated, gave very fair results.

TABLE II (Condensed).

Frog No. 14. Ganglia stimulated, while suspended in normal salt solution, 3½ hours, five stimuli per second, one minute of stimulation alternating with one minute of rest.

Two sets of 100 nuclei each.

	Average Diameters. Mean.	*Remarks.*
9th pair of ganglia, hardened in corrosive sublimate.	Resting.....15.06 Stimulated...13.62 Diff. 1.44	1st set. Measured by myself *previous* to Mr. W.'s measurement of 2d set.
	Resting.....14.34 Stimulated...12.12 Diff. 2.22	2d set. Measured by Mr. W. *without knowledge of my results*, and having but one of the ganglia in field at the same time, and *not knowing which had been stimulated* and which not.
Set 1 and 2.	Resting.....14.70 Stimulated...12.87 Diff. 1.83	

Treating the nuclei as spheres, and computing the volumes from the mean diameters, we have per cent of shrinkage in bulk of nucleus (resting, 100 per cent; stimulated, 67 per cent) 33 per cent.

Differences in staining and appearance of protoplasm not clearly in agreement with those found in frog No. 7 and cat. Treated by Gaule's quadruple staining method, the stimulated cells stained somewhat redder with eosin than the unstimulated. (See remark on staining under Tables III and V.)

The best results were obtained from experiments in which the circulation was kept most normal (see Tables III and V). Table IV gives only a fair showing for frog No. 15, the experiment being not altogether successful.

TABLE III.

Frog No. 7. Made reflex. Stimulated 2½ hours, intervals of rest and stimulation being two minutes.

Three sets of 100 nuclei each. In set 1 the cells were also measured.

		Nuclei. Average of 50 Diameters.			Cells. Diameters.
		Long.	Short.	Mean.	Mean.
8th pair ganglia. Hardened in corrosive sublimate.	Resting.....	16.09	12.50	14.29 1st set.	39.69
	Stimulated ..	13.62	11.47	12.54	35.00
9th pair ganglia.	Resting.....	15.97	12.08	14.03 2d set.	
	Stimulated ..	14.81	10.44	12.62	
2d pair ganglia.	Resting.....	15.78	11.47	13.62 3d set.	
	Stimulated ..	14.25	10.53	12.39	
	Resting.....	15.94	12.02	13.98 Sets 1, 2 and 3.	
	Stimulated ..	14.22	10.81	12.51	

Per cent shrinkage in volume of nucleus, computed as above, 33 per cent (resting, 100 per cent; stimulated, 67 per cent).

It was in this series that the nuclei first appeared shrunken in the stimulated cells.

Staining somewhat lighter in stimulated cells, due to—

1. Protoplasm of stimulated cells less densely and coarsely granular and much vacuolated.

2. Nuclei more distinct in stimulated than in unstimulated cells.

TABLE IV.

Frog No. 15. Cerebrum removed and wound allowed to heal before the experiment. Stimulated 5½ hours at a temperature of + 35° C., intervals of stimulation and rest being one minute.

Three sets of 100 nuclei each.

	Diameters. Mean.		Remarks.
Ganglia of 2d pair, hardened in picric acid.	Resting.....16.94	1st set.	*Set* 1 was measured by myself previous to measurement of second set.
	Stimulated ..16.00		
	Diff. .94		
	Resting.....16.81	2d set.	*Set* 2 was measured by Mr. L. without *any knowledge* as to my own previous measurement and with *no knowledge as to which of the ganglia had been stimulated.*
	Stimulated ..15.47		
	Diff. 1.34		
9th ganglia. Flemming.	Resting.....20.74	3d set.	It will be noted that both Mr. L.'s and Mr. W.'s measurements (Table II) make the difference between stimulated and unstimulated nuclei somewhat greater than my own.
	Stimulated ..19.53		
	Diff. 1.21		
	Resting.....18.16	Sets 1, 2 and 3.	
	Stimulated ..17.00		
	Diff. 1.16		

Volume shrinkage 19 %.
Resting....100 %
Stimulated 81 %

Staining and structure of protoplasm not well defined; probably due to the fact that the frog died toward close of experiment. At its close the muscles were beginning to pass into rigor mortis. Stimuli used excessively strong.

TABLE V.

Cat No. 1. Optic thalami punctured. Stimulated, one minute of stimulation alternating with one minute of rest, for 7 hours.

Two sets of 100 cells and nuclei each.

1st set.	NUCLEI.				CELLS.
	Diameters in mm.				Diameters.
	Long.	Short.	Mean.	Shrinkage.	Mean.
1st dorsal, hardened with osmic acid. Resting....	18.16	14.75	16.45	100%	59.06
Stimulated..	15.84	12.44	14.14	62%	57.19
			Diff. 2.31	38%	

2d set.	Nuclei. Diameters in mm.				Cells. Diameters.
	Long.	Short.	Mean.	Shrinkage.	Mean.
7th cervical, hardened with Flemming. Resting	17.34	15.53	16.44	100%	57.50
Stimulated	16.28	14.37	15.32	80%	56.25
			Diff. 1.12	20%	

Sets 1 and 2.		
Resting	16.44	100%
Stimulated	14.73	71%
Diff.	1.61	29%

The difference between sets 1 and 2 may be due in part to the different hardening agents used. It is probably due in part also to the position of the nerves between the electrodes; the nerve from the 1st dorsal coming first in the circuit, that of the 7th cervical third.

Staining, in general, lighter in the stimulated ganglion.

The experiment on the cat was the most satisfactory of all, both as to operation and results. As anaesthetics are a disturbing factor, the optic thalami were punctured during slight anaesthesia from *ether*. The pulse and respiration remained normal during the whole experiment. The right brachial plexus was laid bare in the axilla and stimulated, one minute of stimulation alternating with an equal time of rest, for seven hours. The cells of the stimulated ganglion show extreme vacuolation, whereas scarcely any is observable in the unstimulated. The nuclei, besides being smaller, are more irregular in outline in the stimulated than in the resting cells. It was noted independently by three observers that the nuclei of the capsule were shrunken in the stimulated cells.

The principal results thus far may be summarized as follows:

1. The nucleus and cell body both decrease in size as a result of stimulation.

2. The protoplasm of the cell becomes vacuolated as a result of stimulation.

3. Differences appear in staining.

These experiments have been made under the guidance of Dr. H. H. Donaldson, Associate in Psychology in the Johns Hopkins University.

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